Richard Sportsman, which in turn claims priority from U.S. Provisional Patent Applications Serial Nos. 60/138,311, filed June 9, 1999; Serial No. 60/138,438, filed June 10, 1999; and Serial No. 60/200,594, filed April 28, 2000; and from U.S. Patent Application Serial No. 09/349,733, filed July 8, 1999, which in turn claims priority from U.S. Provisional Patent Application Serial No. 60/092,203, filed July 9, 1998.--

A1 cont'd

Please replace the paragraph at page 2, lines 1-14 with the following amended paragraph:

--This application also incorporates by reference the following PCT patent applications: Serial No. PCT/US99/01656, filed January 25, 1999; Serial No. PCT/US99/03678, filed February 19, 1999; Serial No. PCT/US99/08410, filed April 16, 1999; Serial No. PCT/US99/16057, filed July 15, 1999; Serial No. PCT/US99/16453, filed July 21, 1999; Serial No. PCT/US99/16621, filed July 23, 1999; Serial No. PCT/US99/16286, filed July 26, 1999; Serial No. PCT/US99/16287, filed July 26, 1999; Serial No. PCT/US99/24707, filed October 19, 1999; Serial No. PCT/US00/00895, filed January 14, 2000; Serial No. PCT/US00/03589, filed February 11, 2000; Serial No. PCT/US00/04543, filed February 22, 2000; Serial No. PCT/US00/06841, filed March 15, 2000; Serial No. PCT/US00/12277, filed May 3, 2000; Serial No. PCT/US00/15774, filed June 9, 2000, entitled *Improvements in Luminescence Polarization Assays*, of inventors J. Richard Sportsman and Lawrence M. Kauvar; and Serial No. PCT/US00/16012, filed June 9, 2000, entitled *Cell-Signaling Assays*, of inventors J. Richard Sportsman and Lawrence M. Kauvar.--

A2

Please replace the paragraph at page 2, line 15 to page 3, line 5 with the following amended paragraph:

--This application also incorporates by reference the following U.S. provisional patent applications: Serial No. 60/138,737, filed June 11, 1999; Serial No. 60/138,893, filed June 11, 1999; Serial No. 60/142,721, filed July 7, 1999; Serial No. 60/143,185, filed July 9, 1999; Serial No. 60/153,251, filed September 10, 1999; Serial No. 60/164,633, filed November 10, 1999; 60/165,813, filed November 16, 1999; Serial No. 60/167,301, filed November 24, 1999; Serial No. 60/167,463, filed November 24, 1999; Serial No. 60/178,026, filed January 26, 2000; Serial No. 60/182,036, filed February 11, 2000; Serial No. 60/182,419, filed February 14, 2000; Serial No. 60/184,719, filed February 24, 2000; Serial No. 60/184,924, filed February 25, 2000; Serial No. 60/190,265, filed March 17, 2000; Serial No. 60/191,890, filed March 23, 2000; Serial No. 60/193,586, filed March 30, 2000; Serial No. 60/197,324, filed April 14, 2000; Serial No. 60/200,530, filed April 27, 2000; and Serial No. 60/202,087, filed May 4, 2000, entitled *Nucleic Acid Detection Methods*, of inventors Susan S. Kalman and Enal S. Ravi.—

A3

Please replace the paragraph at page 28, lines 1-6 with the following amended paragraph:

--The peptide to be coupled to luminescent label was of the formula GEEGYMPMGK (SEQ ID NO: 1), where the tyrosine residue is chemically phosphorylated, and the C-terminal lysine is derivatized on the ε amino group with a biotin. The derivatization with

A4

biotin is irrelevant to the claimed assay. The tracer form of this peptide was prepared by coupling fluorescein to the amino terminus by reaction with fluorescein isothiocyanate (FITC).--

A4 cont

Please replace the paragraph at page 28, lines 10-16 with the following amended paragraph:

--The single-tube reader is a more sensitive instrument, and can use less tracer. In the FPM-1, the tracer form of the peptide (GEEGYMPMGK; SEQ ID NO: 1) coupled with fluorescein to the amino terminus was used at 0.3 nM. To transfer this technology to multiple samples, the assay was transferred to a 96-well format. In doing so, the sensitivity of the instrument is less, and as a result more tracer is required to give adequate signal. We were able to see 7.5 and 15 nM of the tracer form of the peptide coupled with fluorescein. For the simpler tracer, FL-phosphotyramine, we had to use 35 nM of the tracer.--

A5

Please replace the paragraph at page 29, lines 16-21 with the following amended paragraph:

--Several peptides were used as substrates for phosphorylation. These peptides were:

KS2: biotin-EGPWLEEEEEAYGWMDF-amide (SEQ ID NO: 2; Boehringer-Manheim Catalog No. 1768719);

D-peptide: DYMTMQIG (SEQ ID NO: 3);

S-peptide: SRGDYMTMQIG (SEQ ID NO: 4).--

A6

Please replace the paragraph at page 34, line 11 to page 35, line 7 with the following amended paragraph:

--The protein substrate may include a generic substrate capable of being phosphorylated by a variety of S/T kinases. Preferred generic substrates may include histone type III-ss, human/bovine myelin basic protein (MBP), and fragments of MBP including all or part of amino acids 4-14. The lattermost substrate has the following sequence:

EKRPSQRSKYL (SEQ ID NO: 5)

Here (and throughout the application), S is the one-letter abbreviation for serine, T is the one-letter abbreviation for threonine, and E, K, R, . . . are the one-letter abbreviations for other amino acids, as specified in the Appendix. Here, S and T are underlined so that the location(s) of potential phosphorylation sites may be more easily identified. Preferred substrates also may include serine and/or threonine amino acids followed in the C-terminal direction by a proline residue, particularly immediately after (i.e., within one amino acid) of the serine or threonine. Preferred substrates also may include amino acid sequences having one or more serine and/or threonine amino acids, enriched for several amino acids to one or both sides of at least one serine and/or threonine by basic amino acids, such as lysine, arginine, and histidine, particularly within about three amino acids in the C-terminal direction. Basic amino acids, including lysine, arginine, and histidine, generally are positively charged under physiological conditions. These substrates are preferred because they may have enhanced recognition by kinase enzymes.--

Please replace the paragraph at page 36, line 6 to page 37, line 2 with the following amended paragraph:

--Preferred specific binding partners include immunological specific binding partners, such as antibodies. In particular, preferred specific binding partners include antibodies against unphosphorylated or phosphorylated forms of the preferred protein substrates listed above, including antibodies against the following phosphorylated MBP fragments:

EKRPpSRSKYL (SEQ ID NO: 6)

EKRPSQRpSYL (SEQ ID NO: 7)

EKRPpSRpSYL (SEQ ID NO: 8)

A8

Here, a "p" is used to denote phosphorylation of the subsequent amino acid. Preferred specific binding partners also include antibodies against the following phosphorylated peptides:

KRREILSRRPpSYRK (SEQ ID NO: 9)
KHFPQFpSYSAS (SEQ ID NO: 10)
pSPELERLIIQC (SEQ ID NO: 11)
GSPSVRCSpSMpS (SEQ ID NO: 12)
RSRHSpSYPAGT (SEQ ID NO: 13)
LpTPLK (SEQ ID NO: 14)
FpTPLQ (SEQ ID NO: 15)
RKRpTLRRL (SEQ ID NO: 16)
LRRApSLG (SEQ ID NO: 17)
KKLNRTLpSVASL (SEQ ID NO: 18)
RPRAApTF-NH_2 (SEQ ID NO: 19)
LRRApSLG-NH_2 (SEQ ID NO: 20)

The lattermost two peptides are amidated at the C-terminal, which may act to stabilize the peptides.--

Please replace the paragraph at page 46, lines 3-13 with the following amended paragraph:

--In still another aspect, the invention is directed to specifically designed peptides that show enhanced affinity for binding to the 4G10 antibody, which is directed to phosphorylated tyrosine. Phosphorylated tyrosine is an indicator of an activated form of the insulin receptor and many other receptors such as EGF-receptor, and IGF-1 receptor, as well as nonreceptor tyrosine kinases such as src, fyn and many others known in the art. These peptides are of the formula

A9

FL-A-pY-TGLSTRNQET-pY-ATH-NH_2 (SEQ ID NO: 21)

FL-pY-pY-pY-IE-NH_2 (SEQ ID NO: 22)

FL-G-pY-NELNLGRREE-pY-DVL-NH_2 (SEQ ID NO: 23)

Here, Y is the one-letter abbreviation for tyrosine, pY is phosphorylated tyrosine, and A, T, G . . . are one-letter abbreviations for other amino acids, as specified in the Appendix.--

Please replace the paragraph at page 50, lines 14-16 with the following amended paragraph:

--The macromolecule loaded with Ga^{3+} (MM-Ga) was tested for the binding of the following di-(phosphotyrosine) peptide fluorescein-labeled tracer, denoted tyrosine kinase 1 (TK-1) tracer: FL-A-pY-TGLSTRNQET-pY-ATH-NH_2 (SEQ ID NO: 21).--

A10

Please replace the paragraph at page 51, lines 7-9 with the following amended paragraph:

--The macromolecule loaded with Ga^{3+} (MM-Ga) also was tested for the binding of the following fluorescein-labeled monophosphoserine peptide tracer, denoted serine/threonine kinase 1 (STK-1) tracer: FL-RFARKG-pS-LRQKNV (SEQ ID NO: 24).--

A11

Please replace the paragraph at page 51, line 18 to page 52, line 2 with the following amended paragraph:

--We demonstrated the specificity of the binding between phosphorylated peptide and a macromolecule activated with Ga (III) metal ion (MM-Ga) and its potential in the development of generic kinase assays. The materials included cAMP-dependent protein kinase A (PKA, from Promega) as the enzyme and fluorescein-labeled Kemptide (fluo-Leu-Arg-Arg-Ala-Ser-Leu-Gly; SEQ ID NO: 25) as the substrate.--

A12

Please replace the paragraph at page 55, line 17 to page 56, line 3 with the following amended paragraph:

--This example shows assays for integrated cell signaling mechanisms. Specifically, the assays described here for kinase and phosphatase enzymes may be performed in combination with the assays for cyclic nucleotides and GTP-binding proteins described in PCT Patent Application Serial No. PCT/US00/16012, filed June 9, 2000, entitled *Cell-Signaling Assays*, of inventors J. Richard Sportsman and Lawrence M. Kauvar, which is incorporated herein by reference. Such combination assays permit study of signaling mechanisms involving multiple pathways.--

A13

Please replace the paragraph at page 64, line 2-21 with the following amended paragraph:

--13. The method of paragraph 1, wherein the peptide is selected from the group consisting of

ApYTGLSTRNQETpYATH-NH_2 (SEQ ID NO: 26),

pYpYpYIE-NH_2 (SEQ ID NO: 27),

GpYNELNLGRREEpYDVL-NH_2 (SEQ ID NO: 28),

EKRPpSRSKYL (SEQ ID NO: 29),

EKRPSQRpSYL (SEQ ID NO: 30),

EKRPpSRpSYL (SEQ ID NO: 8),

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KRREILSRRPpSYRK (SEQ ID NO: 9),

KHFPQFpSYSAS (SEQ ID NO: 10),

pSPELERLIIQC (SEQ ID NO: 11),

GSPSVRCSpSMpS (SEQ ID NO: 12),

RSRHSpSYPAGT (SEQ ID NO: 13),

LpTPLK (SEQ ID NO: 14),

FpTPLQ (SEQ ID NO: 15),

RKRpTLRRL (SEQ ID NO: 16),

LRRApSLG (SEQ ID NO: 17),

KKLNRTLpSVASL (SEQ ID NO: 18),

RPRAApTF-NH_2 (SEQ ID NO: 19), and

LRRApSLG-NH_2 (SEQ ID NO: 20).--

Please replace the paragraph at page 66, lines 17-19 with the following amended paragraph:

--24. The method of paragraph 23, wherein the modified form of the substrate is the phosphorylated form of biotin-EGPWLEEEEEAYGWMDF-amide (KS2-peptide; SEQ ID NO: 2), DYMTMQIG (D-peptide; SEQ ID NO: 3), or SRGDYMTMQIG (S-peptide; SEQ ID NO: 4).--

A15

Please replace the paragraph at page 67, lines 7-20 with the following amended paragraph:

--27. A method to determine the presence and amount of a posttranslationally modified protein or peptide comprising, contacting a sample containing said posttranslationally modified protein or peptide with a luminescence tracer and a specific binding partner, wherein said tracer and said posttranslationally modified protein or peptide compete for binding to said partner, and wherein, when said tracer is bound to said partner, its luminescence polarization is changed from that of the tracer in unbound form, followed by measuring the luminescence polarization in the sample, whereby a change in the luminescence polarization to more closely approximate that of the tracer in unbound form is a measure of the presence or amount of said posttranslationally modified protein or peptide, wherein the posttranslational modification results in a posttranslationally modified substrate from an enzyme catalyzed reaction of a protein or peptide substrate that is catalyzed by the insulin receptor cytoplasmic kinase domain (IR-CKD) or IGF-1r cytoplasmic kinase domain (IGF-1r-CKD), and wherein the tracer is a fluorescein conjugate of the peptide GEEGYMPMGK (SEQ ID NO: 1) or N-acetyl-O-phosphotyramine.--

A16